Trk Signaling Pathway

Effect of ARQ 531 on Trk Pathway

ARQ 531 potently inhibits TrkA, B and C

ARQ 531 potently inhibits Trk family kinases biochemically and cellularly

ARQ 531 potently inhibited cell proliferation driven by Trk fusion kinases

ARQ 531 markedly suppresses phosphorylation of TPM3-TrkA fusion kinase and its downstream targets

ARQ 531 markedly suppresses pPLCγ1, pAKT, and pERK and induces apoptotic response

ARQ 531 significantly reduced tumor weight in comparison to vehicle treated group

In Vivo Efficacy of ARQ 531 in Trk Fusion-driven KM12 Xenograft Model

Mice in treated groups showed slight weight loss within in the first 3 days but recovered during treatment

In Vivo and In Vitro Effect of ARQ 531 on Trk Family Kinases

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RESULTS

At concentrations of ARQ 531 and the IC50 for Ba/F3(ETV6-NTRK2) were determined using the PathHunter technology. The PathHunter U2OS cells expressing TrkA, TrkB or TrkC were treated with various concentrations of ARQ 531 for 10 minutes. pTrkA, pAKT and pERK were assessed using Western blot analysis.

ARQ 531 potently suppressed tumor growth with TGI of 51%, 61%, and 66%, respectively.

In Vivo Anti-tumor activity was assessed by determining tumor volumes. Mice were inoculated with KM12-Luc cells and orally administered ARQ 531. ARQ 531 significantly reduced tumor weight in comparison to vehicle treated group.

MATERIALS AND METHODS

Cells and Cell Culture

K-562 cells (PMN TRK A, B and C) stably expressed TrkA, B and C were determined as an ATP concentration equal to its Km value. Cell-based kinase functional assay was performed using the PathHunter technology. The PathHunter U2OS cells expressing TrkA, TrkB or TrkC were treated with various concentrations of ARQ 531 for 10 minutes, and then stimulated with NGF at 100 ng/mL for 30 minutes. pTrkA, pAKT and pERK were assessed using Western blot analysis.

Western Blot Analysis

Cells were maintained in humidified atmosphere with 5% CO2 and 95% humidified atmosphere with 5% CO2 and treated with NGF at 100 ng/mL or forskolin at 10 μM. The IC50 of forskolin was assessed. pTrkA, pPLCγ1, pAKT, and pERK were examined using Western blot analysis and their expression levels were measured at 30 minutes using a TLR4 specific probe. The IC50 was determined using GraphPad Prism. The proliferation assays for Ba/F3 expressing ETV6-NTRK2, -NTRK1 or -NTRK3 were performed using a CellTiter-Glo assay at Advanced Cellular Dynamic/Carnabiosciences.

ARQ 531 significantly reduced tumor weight in comparison to vehicle treated group.

CONCLUSIONS

ARQ 531 potently inhibited Trk family kinases, as demonstrated in biochemical and cell-based assays.

ARQ 531 improved the Trk signal pathway in TrkA overexpressing K-562 cells and TPM3-TrkA fusion KM12 cells.

ARQ 531 potently inhibited proliferation of TPM3-TrkA fusion driven KM12 cells and Ba/F3 cells with ETV6-TrkA, -TrkB, and –TrkC.

In a KM12 xenograft mouse model, ARQ 531 exhibited marked anti-tumor activity with a significant reduction of tumor volume and tumor weight.

The data provide a rationale for testing ARQ 531 in cancer patients with Trk fusion kinases.